

Entrapment of Chromosomes by Condensin Rings Prevents Their Breakage during Cytokinesis

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SUMMARY

Successful segregation of chromosomes during mitosis and meiosis depends on the action of the ring-shaped condensin complex, but how condensin ensures the complete disjunction of sister chromatids is unknown. We show that the failure to segregate chromosome arms, which results from condensin release from chromosomes by proteolytic cleavage of its ring structure, leads to a DNA damage checkpoint-dependent cell-cycle arrest. Checkpoint activation is triggered by the formation of chromosome breaks during cytokinesis, which proceeds with normal timing despite the presence of lagging chromosome arms. Remarkably, enforcing condensin ring reclosure by chemically induced dimerization just before entry into anaphase is sufficient to restore chromosome arm segregation. We suggest that topological entrapment of chromosome arms by condensin rings ensures their clearance from the cleavage plane and thereby avoids their breakage during cytokinesis.

INTRODUCTION

Condensin complexes play central roles in the successful segregation of chromosomes during mitotic and meiotic cell divisions (reviewed by [Piazza et al., 2013](#)). Although the effects of depletion or mutation of condensin subunits on the proper folding of mitotic chromosomes vary between model systems, chromosomes that lack condensin predominantly fail to partition correctly during anaphase (reviewed by [Hirano, 2012](#)). The molecular mechanisms by which condensin prevents lagging or bridged chromosomes have remained poorly understood. One option is that condensin, together with topoisomerase II (topo II), actively participates in the decatenation of entangled sister chromatids and thereby enables their complete separation ([Baxter et al., 2011](#)). This function might be particularly important at repetitive chromosome regions such as, for example, the ribosomal DNA repeats in budding yeast ([D'Ambrosio et al., 2008](#);

[D'Amours et al., 2004](#)). Alternatively, condensin might be required to generate a recoiling force that promotes the removal of residual sister chromatid cohesion ([Renshaw et al., 2010](#)). Yet another hypothesis envisions that condensin functions as a molecular linker that connects different parts of a chromosome and thereby assists in chromatid resolution (discussed in [Cuylen and Haering, 2011](#)).

Surprisingly little is known about how cells deal with the chromosome segregation failures that result from deficiencies in condensin function. Lagging or nondisjoined chromosomes present evidently an obstacle for cytokinesis. In animal cells, chromosome bridges frequently result in the abortion of cytokinesis and the formation of tetraploid cells ([Mullins and Bieseke, 1977](#); [Shi and King, 2005](#)), an event that has been shown to promote tumorigenesis ([Fujiwara et al., 2005](#)). Recent work demonstrated that Aurora B kinase activity delays abscission in cells with chromosome bridges, most likely to allow resolution of the bridges and to prevent tetraploidization resulting from cleavage furrow regression ([Carlton et al., 2012](#); [Steigemann et al., 2009](#)). Condensin I, which colocalizes with Aurora B to chromosome bridges, might play a crucial role in this process ([Bembenek et al., 2013](#)). Despite this surveillance mechanism, chromosomes that failed to segregate get frequently damaged during cytokinesis ([Gascoigne and Cheeseman, 2013](#); [Janssen et al., 2011](#)). A similar Aurora kinase-dependent mechanism has been suggested to prevent severance of nonsegregated chromosomes during cytokinesis in budding yeast ([Mendoza et al., 2009](#); [Norden et al., 2006](#)). Other studies, however, report that cell division proceeds even in the presence of nondisjoined chromatin masses in budding ([Baxter and Diffley, 2008](#); [McGrew et al., 1992](#)) and fission yeasts (to produce a so-called cut phenotype; [Hirano et al., 1986](#)).

We recently discovered that condensin complexes topologically entrap chromosomal DNAs within the large ring-shaped structure formed by their structural maintenance of chromosomes (Smc2–Smc4) and kleisin (Brn1) subunits ([Cuylen et al., 2011](#)). Release of condensin from chromosomes in live budding yeast cells by ring opening through site-specific cleavage of Brn1 with tobacco etch virus (TEV) protease has no effect on the partitioning of the centromeres of a model chromosome (chromosome V) but frequently prevents the segregation of the arm of the same chromosome into the daughter cell. Here, we investigate the consequences that arise from the persistence

of chromosome arms at the cytokinetic cleavage plane after condensin release.

RESULTS

Condensin Cleavage Induces a DNA Damage Checkpoint-Dependent Cell-Cycle Arrest

To investigate the consequences of chromosome arm segregation failure after condensin release from chromosomes, we opened condensin rings by TEV-mediated Brn1 cleavage in yeast cells that had been arrested in metaphase by depletion of the anaphase-promoting complex/cyclosome (APC/C) activator Cdc20. We then released the cells from the arrest by Cdc20 reinduction and recorded their progression through the cell cycle by live-cell microscopy in a microfluidic flow chamber for more than 12 hr. Surprisingly, despite chromosome segregation defects, more than 85% of cells with cleaved condensin rings exited mitosis and entered a new cell cycle (Figure 1A). However, nearly all of these cells then arrested as large-budded cells for the remainder of the time course (Figure 1B). Cells with intact condensin rings, in contrast, typically underwent five to six divisions during the period of imaging (Figure 1A). This suggests that most cells that undergo mitosis with cleaved condensin rings are blocked in the following G2 or M phase.

To determine more precisely at which stage of the cell cycle cells with cleaved condensin rings arrest, we monitored mitotic spindle dynamics in cells expressing GFP-labeled tubulin. Cells with intact Brn1 underwent anaphase within ~30 min (Figure 1C; Movie S1 available online) and entered the next cell cycle within ~60 min after release from metaphase, as judged by bud formation (Figure 1D). Ninety-five percent of these cells then assembled a new metaphase spindle and initiated a second anaphase within 190 min after release (Figure 1E). The majority of cells released from the metaphase arrest with cleaved condensin rings completed the first anaphase, entered the next cell cycle, and assembled a new metaphase spindle with a similar timing as cells with intact condensin rings. However, these cells then failed to initiate a second anaphase and instead remained arrested with a metaphase spindle until the end of the time course (Figures 1D–1F; Movie S1).

A plausible cause for the arrest in metaphase might be a defect in the attachment of kinetochores to mitotic spindle microtubules after condensin release from chromosomes (Brito et al., 2010). Unattached kinetochores are sensed by the spindle assembly checkpoint (SAC), which prevents anaphase onset by inhibiting the APC/C (Musacchio and Salmon, 2007). We reasoned that if activation of the SAC were responsible for the metaphase arrest, deletion of the gene encoding the central SAC component Mad2 should override the arrest. However, *mad2Δ* cells still failed to undergo a second anaphase when condensin had been released from chromosomes during the previous mitosis (Figure 1G).

An alternative pathway that blocks budding yeast cells in metaphase is the Mec1/Rad9-dependent DNA damage checkpoint (Weinert and Hartwell, 1988). Notably, deletion of the gene encoding Rad9 allowed approximately half of the cells that had assembled a new metaphase spindle after condensin ring cleavage to enter a second anaphase (Figures 1H and 1I; Movie S2). Segregation of chromosomes without condensin

rings attached to them might therefore result in the formation of DNA breaks, which then trigger a DNA damage checkpoint-dependent arrest in metaphase of the next cell cycle.

Chromosomes Depleted of Condensin Break during Cytokinesis

To test for the presence of DNA breaks after condensin release from chromosomes, we imaged cells that express a GFP fusion construct of the DNA double-strand break-repair protein Rad52, which forms nuclear foci in response to DNA damage (Figure S1A). We cleaved condensin's Brn1 subunit in cells arrested in metaphase, released the cells from the arrest, and monitored formation of Rad52-GFP foci by fluorescence microscopy (Figures 2A–2C). Strikingly, more than 70% of cells with cleaved condensin rings accumulated at least one Rad52-GFP focus within 2.5 hr after the release from metaphase. In contrast, Rad52-GFP foci were detectable on average in only 10% of cells with intact condensin rings. The fraction of cells with cleaved condensin rings that displayed Rad52-GFP foci increased notably 90 min after release from the metaphase arrest, at a time when most cells were passing through S phase (Figure 2A). DNA breaks might therefore arise during replication of chromosomes devoid of intact condensin rings. Alternatively, breaks could have occurred earlier, but might not have accumulated Rad52 until later in the cell cycle (Lisby et al., 2004).

To test whether DNA breaks form before entry into S phase, we repeated the experiment, but this time, following condensin ring cleavage during the metaphase arrest, released cells into media containing α -factor mating pheromone to rearrest them in G1 phase. In this case, we did not detect an increase of Rad52-GFP foci in cells with cleaved condensin rings (Figures S1B–S1D), most likely because Rad52 does not efficiently form foci in cells arrested in G1 phase (Figure S1E; Barlow et al., 2008). To be able to detect DNA breaks during G1 phase, we used cells that express the DNA double-strand break-repair protein Mre11 fused to GFP. Notably, we detected at least one Mre11 focus in more than 35% of cells with cleaved condensin within 60 min of release into the G1 phase arrest, whereas Mre11-GFP foci formed in less than 5% of cells with intact condensin rings (Figures 2D–2F). We conclude that DNA breaks occur before the onset of DNA replication if cells complete mitosis in the absence of chromosome-bound condensin.

Two possibilities could explain the observed chromosome breakage. First, mitotic spindle forces might rupture chromosomes that lack condensin during segregation, and the breaks might somehow escape detection by the anaphase DNA damage checkpoint (Yang et al., 1997). Second, chromosome arms that fail to segregate in the absence of intact condensin and remain at the cleavage plane might be severed during cytokinesis. To distinguish between these two possibilities, we tested whether DNA breaks can be detected after anaphase chromosome segregation but before cytokinesis. Following condensin ring opening in cells arrested in G1 phase, we released cells into the cell cycle and then rearrested them in late anaphase by inactivation of the mitotic exit network component Cdc15 (Jaspersen et al., 1998) (Figures 2G and S1F). Even though most *cdc15-2* cells had segregated their chromosome masses by 120 min after release from G1 phase (Figure S1G), we did not detect an increase in the number of cells with Mre11-GFP

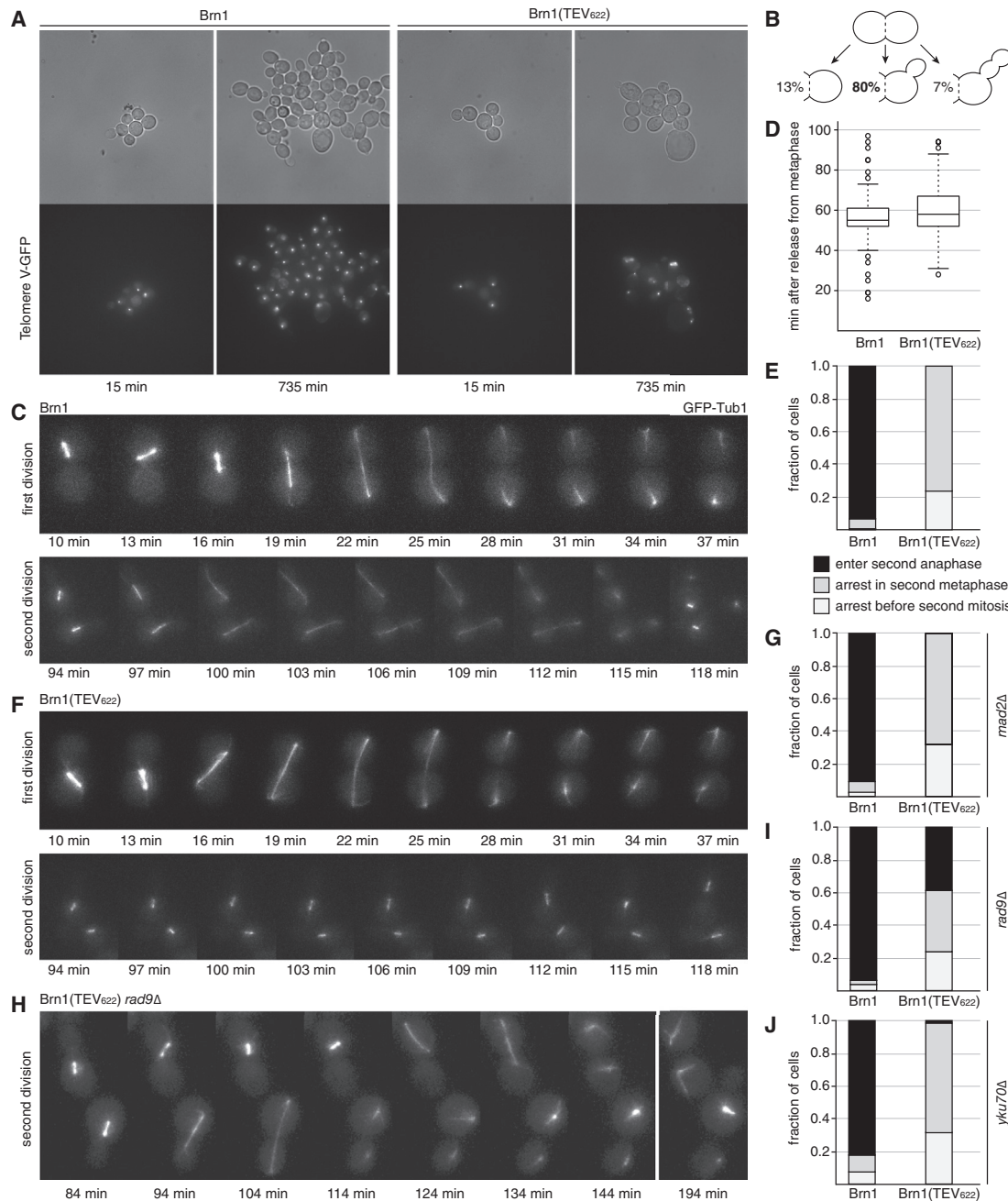


Figure 1. Condensin Release from Chromosomes Results in a DNA Damage Pathway-Dependent Cell-Cycle Arrest

(A and B) Cell division and segregation of a GFP marker array integrated proximal to the right telomere of chromosome V were monitored for 12.25 hr in a microfluidic flow cell of cells released from metaphase with intact (strain C2513) or TEV-cleaved (strain C2618) Brn1. (A) Example images from 15 and 735 min time points are shown. (B) The fraction of cells that did not form buds, budded once, or rebudded a second time was scored ($n = 69$ cells). (C) Mitotic spindle dynamics were recorded of a cell released from metaphase with intact condensin (strain C3257). (D) The time between release from metaphase arrest and bud formation was scored for cells with intact or cleaved condensin (strains C3257 and C3258). Data represent the median (line), the 25th, 75th (boxes), 5th, and 95th percentiles (whiskers) ($n = 69$ –114 cells). (E) The fractions of cells from (C) and (F) that failed to form a new mitotic spindle, arrested with a metaphase spindle, or underwent a second anaphase spindle extension within 190 min of release from metaphase were scored ($n \geq 130$ cells). (F) Mitotic spindle dynamics of a cell with cleaved Brn1 (strain C3258) was monitored as in (C). (G) Passage through mitosis of *mad2Δ* cells (strains C3279 and C3280) was scored as in (E) ($n > 110$ cells). (H) Mitotic spindle dynamics of a *rad9Δ* cell with cleaved Brn1 (strain C3512) was monitored as in (C). (I) Passage through mitosis of *rad9Δ* cells (strains C3467 and C3512) was scored as in (E) ($n > 120$ cells). (J) Passage through mitosis of *yku70Δ* cells (strains C3873 and C3874) was scored as in (E) ($n > 95$ cells).

See also [Movies S1](#) and [S2](#).

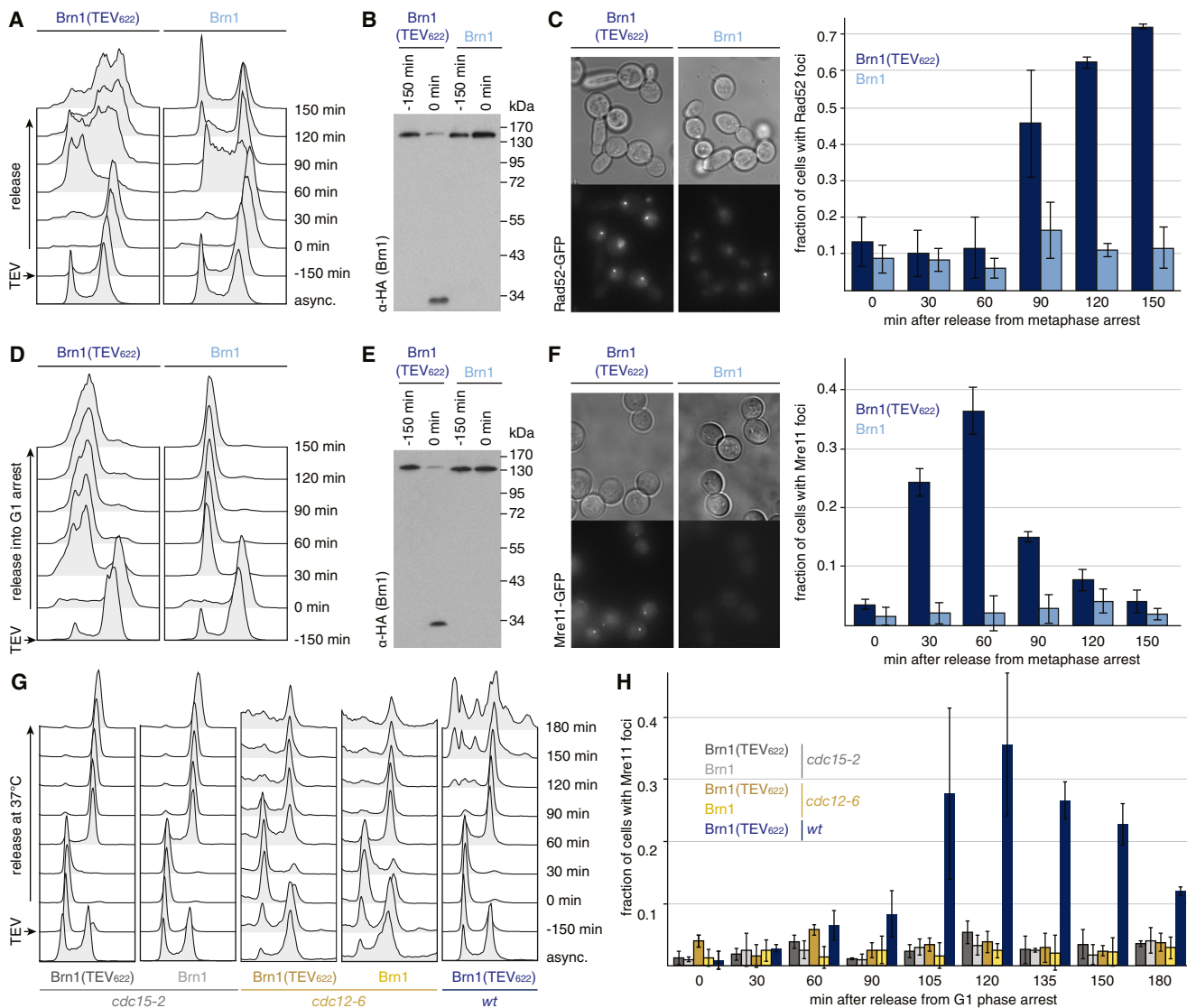


Figure 2. DNA Breaks Occur after Mitotic Exit and before Entry into the Next S Phase

(A–C) Cells were released from metaphase with cleaved or intact Brn1 (C3310 and C3308) by Cdc20 reexpression. (A) Cell-cycle progression was scored by FACScan analysis of cellular DNA content. (B) Brn1 cleavage was monitored by western blotting against the C-terminal HA₆ tag. (C) Rad52-GFP foci formation was scored at the indicated time points. Example images from the 120 min time point are shown.

(D–F) Cells were released with cleaved or intact Brn1 (strains C3380 and C3382) into media containing α factor to rearrest them in G1 phase. (D) Cell-cycle progression was monitored as in (A). (E) Brn1 cleavage was monitored as in (B). (F) Mre11-GFP foci formation was scored at the indicated time points. Example images from the 30 min time point are shown.

(G and H) TEV protease expression was induced in cells synchronized in G1 phase by α factor. Cells were released at 37°C to rearrest them in late anaphase as a result of *cdc15* inactivation (strains C3432 and C3434) or to prevent cytokinesis in the *cdc12* mutant (strains C3801 and C3796) and were compared to *CDC12/CDC15* control cells (strain C3381). Cell-cycle progression and DNA break formation were monitored as in (D) and (F) at the indicated time points.

Data in (C), (F), and (H) represent the mean of triplicate experiments \pm SD ($n > 35$ cells per time point and experiment).

See also Figure S1.

foci (Figure 2H). In contrast, more than 35% of *CDC15* wild-type cells with cleaved condensin rings displayed at least one Mre11-GFP focus within 120 min of release from G1 phase. Hence, exit from mitosis is required to induce DNA breakage in cells that lack condensin bound to chromosomes. Finally, we repeated the experiment in septin mutant *cdc12-6* cells (Adams and Pringle, 1984), which are unable to complete cytokinesis. Consistent with the hypothesis that DNA breaks are

caused by cleavage of chromosome during cytokinesis, we observed no increase in the formation of Mre11-GFP foci in the septin mutants (Figure 2H).

Nonsegregated Chromosomes Do Not Inhibit Cell Division

The finding that chromosome breaks occur only in cells that undergo cytokinesis suggests that, in the absence of

chromosome-bound condensin, yeast cells divide despite the presence of nonsegregated chromosomes at the cleavage plane. To directly test whether cell division proceeds, we recorded simultaneously mitotic spindle and actomyosin ring dynamics in cells expressing the α -tubulin subunit Tub1 tagged with GFP and the myosin motor subunit Myo1 tagged with mCherry after release from metaphase with intact or cleaved condensin (Figures 3A–3B; Movie S3). Actomyosin contraction in cells with intact condensin commenced 8.6 ± 1.5 min after the onset of anaphase spindle extension (Figure 3C) and was completed after another 4.2 ± 0.7 min (Figure 3D). Timing was only marginally slower in cells with cleaved condensin, where actomyosin ring contraction started 8.7 ± 1.7 min after anaphase onset and was completed after another 4.4 ± 1.0 min. Using the same assay, we compared the localization dynamics of chitin synthase Chs2 (Figures 3E–3F; Movie S3), which is delivered to the bud neck for septum formation (Chuang and Schekman, 1996). Chs2 appeared at the bud neck 9.2 ± 1.6 min after anaphase onset in cells with intact condensin and 9.5 ± 1.7 min after anaphase onset in cells with cleaved condensin (Figure 3G). Chs2 signals at the bud neck decreased as the actomyosin ring contracted and had almost completely disappeared 8.3 ± 1.5 min or 8.9 ± 1.7 min later in cells with intact or cleaved condensin rings, respectively (Figure 3H).

Although the timing of cytokinetic events appears not to be considerably affected in cells that missegregate chromosomes because of condensin release, it is nevertheless conceivable that these cells might fail to physically separate. To test this possibility, we adapted a live-cell microscopy assay that had been previously used in mammalian cells to probe for the completion of cytokinesis by measuring the cytoplasmic continuity between postmitotic daughter cells (Steigemann et al., 2009). We briefly illuminated with a laser a small volume in one daughter half of dividing yeast cells expressing the GFP-variant EosFP (Wiedenmann et al., 2004) to photoconvert the emission spectrum of the EosFP molecules in this volume from green to red. If the two daughter cells were still connected, the red fluorescence signal in both daughters should rapidly equalize as a result of diffusion of the photoconverted EosFP (Figure 3I). In contrast, only one of the two daughters should display red fluorescence if cell division had been completed.

To validate the assay, we first photoconverted EosFP in one of the two daughter halves of cells arrested in metaphase. As expected, the red fluorescence signal rapidly diffused into the other daughter half (Figure 3J; Movie S4), and red fluorescence intensities in the two cell halves were almost equal 10 s after photoconversion (Figure 3K). We next released cells with cleaved or intact condensin rings (Figure S2A) from the metaphase arrest and only photoconverted EosFP in one of the two daughters after cells had entered the next cell cycle, as evident by the formation of a bud in at least one of the two daughter cells. Strikingly, diffusion of the red EosFP signal was now restricted to only the daughter cell in which EosFP had been photoconverted (Figures 3J–3K; Movie S4). This experiment suggests that cells are able to complete cell division even when condensin had been released from chromosomes during the preceding metaphase. To rule out that diffusion of EosFP into the other daughter cell might merely be very slow, possibly because of the persistence of only small connections between the daughter

cells, we imaged cells for 90 min after photoconversion. Still, we were not able to detect an increase in red fluorescence in the daughter cell that had not been photoconverted (Figure S2B). Finally, we cleaved condensin already during G1 phase, released cells into the cell cycle, and then photoconverted EosFP at the distal end of the elongated daughter cell once cells had formed a new bud. Although we were able to follow the rapid diffusion of photoconverted EosFP along the axis of the cell, we could not detect an increase in red fluorescence in the other daughter (Figures 3J–3K; Movie S4). We conclude that the chromosome segregation failures that result from condensin release from chromosomes do not prevent the completion of cell division.

It has been suggested that the presence of nonsegregated chromosomes at the plane of cell division is sensed by the NoCut checkpoint pathway, which delays completion of cytokinesis to prevent chromosome breakage (Mendoza et al., 2009; Norden et al., 2006). The finding that cells divide after condensin cleavage despite the presence of lagging chromosomes raises the possibility that condensin might be part of a pathway that links chromosome segregation to cytokinesis (Bembenek et al., 2013). We therefore tested whether cell division measured by our assay is inhibited when we prevent chromosome segregation in the presence of intact condensin. First, we overexpressed in cells arrested in G1 phase a version of the cohesin subunit Scc1 that cannot be cleaved by separase (Figure S2C). When released from the G1 arrest, cells fail to resolve cohesion and attempt cell division without sister chromatid separation (Uhlmann et al., 1999). In these cells, we could still not detect diffusion of EosFP from one daughter cell into the other (Figure 3K), despite severe chromosome segregation failures (Figure S2C). Second, we inhibited sister chromatid resolution by inactivation of topo II, which causes the bulk of chromatin to span across the bud neck (Figure S2D). Again, we did not detect diffusion of EosFP between the daughter cells (Figure 3K). In summary, our results demonstrate that cell division can proceed to a level that prevents diffusion of a small protein even when nonsegregated chromosomes are present at the cleavage plane, independent of whether condensin is bound to these chromosomes.

Condensin Ring Integrity Is Sufficient to Allow Chromosome Arm Segregation

Our experiments suggest that the failure to segregate chromosomes during anaphase results from the release of condensin from chromosomes by ring opening. We wondered whether restoring the ring integrity of cleaved condensin complexes at the metaphase-to-anaphase transition might then be sufficient to restore condensin function. We engineered into Brn1 TEV sites flanked by two repeats of the human FKBP12 and Frb proteins, which, in the presence of the small molecule rapamycin, form stable heterodimers (Gruber et al., 2006). Addition of rapamycin after condensin ring opening by TEV cleavage should therefore allow condensin ring reclosure (Figure 4A).

We first tested whether rapamycin-induced dimerization is sufficiently strong to maintain the function of condensin rings after Brn1 cleavage. We induced TEV protease expression in asynchronous cells that express Brn1 with FKBP12- and Frb-flanked TEV sites inserted into one of two different positions

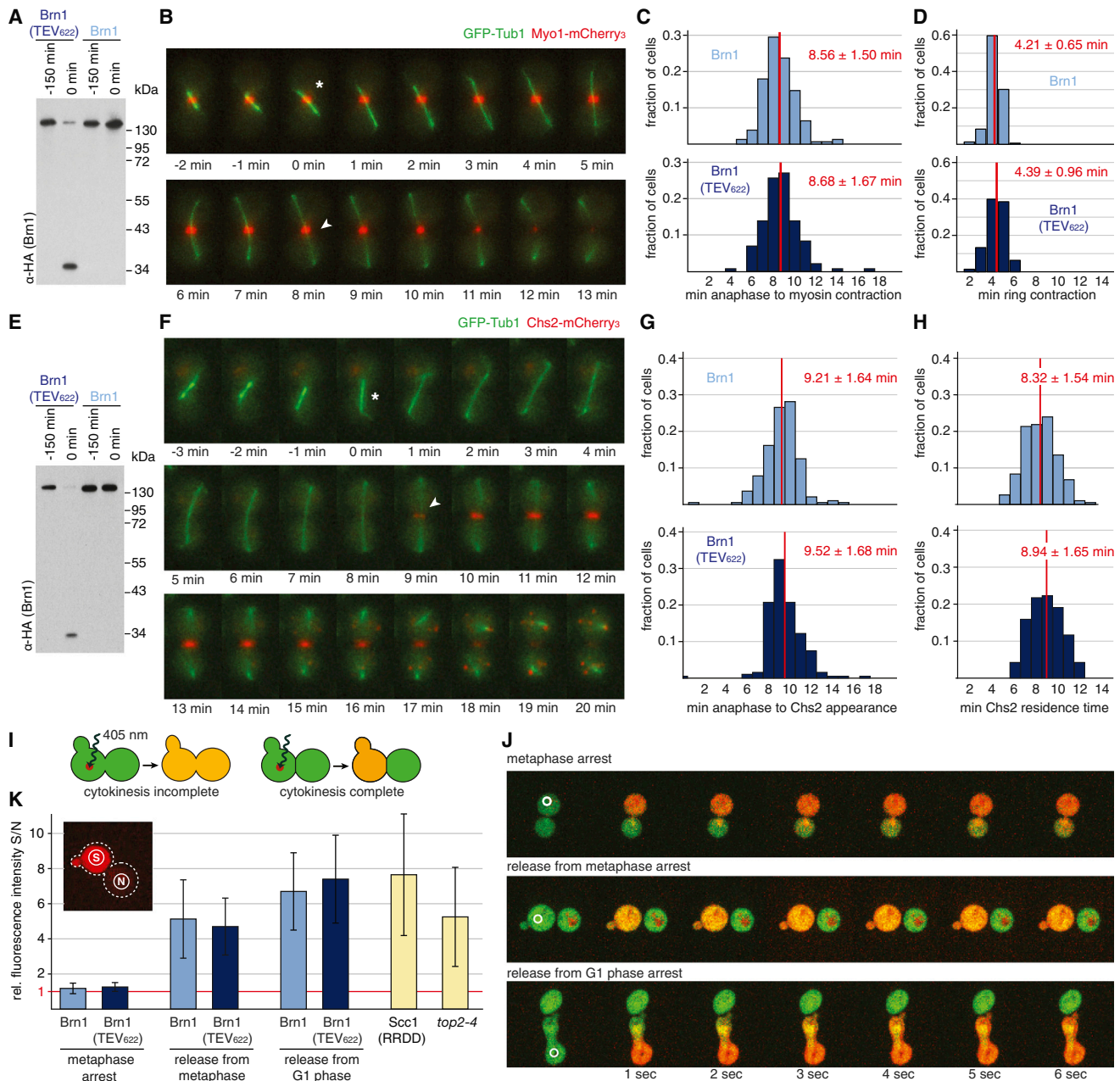


Figure 3. Cells Complete Division after Condensin Release from Chromosomes

(A–D) TEV protease expression was induced in metaphase-arrested cells expressing TEV-cleavable or noncleavable Brn1 (strains C3875 and C3898). Cells were released from the metaphase arrest by Cdc20 reexpression and actomyosin ring contraction (Myo1-mCherry₃), and mitotic spindle dynamics (GFP-Tub1) were monitored. (A) Western blotting against the C-terminal HA₆ tag on Brn1. (B) Representative image sequence of a cell expressing TEV-cleavable Brn1. Anaphase onset (asterisk) and start of actomyosin contraction (arrowhead) are indicated. Histogram plots of time (C) from anaphase onset to initiation of actomyosin ring contraction and (D) from initiation of actomyosin ring contraction to Myo1 disappearance from the bud neck ($n > 140$ cells; mean \pm SD).

(E–H) Chs2 localization dynamics (Chs2-mCherry₃) and mitotic spindle dynamics (GFP-Tub1) were monitored in cells expressing TEV-cleavable or noncleavable Brn1 (strains C3899 and C3877) as in (A)–(D). (E) Brn1 cleavage was monitored as in (A). (F) Representative image sequence of a cell expressing TEV-cleavable Brn1. Anaphase onset (asterisk) and appearance of Chs2 at the bud neck (arrowhead) are indicated. Histogram plots of time (G) from anaphase onset to Chs2 appearance and (H) from Chs2 appearance and disappearance at the bud neck ($n > 180$ cells; mean \pm SD) are shown.

(I–K) EosFP was photoconverted from green to red fluorescence in a small area in one daughter half of cells expressing noncleavable or TEV-cleavable Brn1. (I) Diffusion of photoconverted EosFP would rapidly equalize the fluorescence spectra of both daughters if they were still connected (left), but not if cytokinesis had been completed (right). (J) Image sequences after photoconversion of EosFP (white circles) after Brn1 TEV cleavage in cells arrested in metaphase (top) or released from the metaphase arrest (middle; strain C3099) or in cells released from G1 phase (bottom; strain C3246). (K) Red fluorescence excitation intensities in photoconverted (switched, S) and nonconverted (nonswitched, N) daughter cells were measured 10 s (metaphase arrest and release; strains C3099 and C3100).

(legend continued on next page)

and plated cells onto media with or without rapamycin. Although these cells failed to grow in the absence of rapamycin, they proliferated indistinguishable from cells that express noncleavable Brn1 in the presence of rapamycin (Figure 4B). Keeping condensin rings closed by FKBP12-Frb-mediated dimerization is hence sufficient to maintain condensin function.

We next tested whether ring reclosure at different cell-cycle stages could restore condensin function. First, we cleaved Brn1 in cells arrested in G1 phase and then plated them immediately (Figure S3A) or after allowing them to progress into a nocodazole-induced mitotic arrest (Figure S3B) onto media with or without rapamycin. In both cases, cells grew comparable to cells with intact condensin on media with rapamycin but failed to grow on media without rapamycin. To assay whether reclosure of condensin rings rescues chromosome segregation defects, we cleaved Brn1 in cells arrested in G1 phase (Figure S3C), then released cells from the arrest into media with or without rapamycin, and monitored segregation of a fluorescent marker array integrated at the distal end of the long arm of chromosome V by time-lapse microscopy (Figures 4C and 4D). In the absence of rapamycin, only 26% of cells with cleaved condensin rings successfully segregated the labeled chromosome arm into the two daughter cells within 165 min after release from the G1 phase arrest. Addition of rapamycin by the time of release increased the fraction of cells with successfully segregated chromosome V arms to 61% (Figure 4D). Rapamycin had no notable effect on chromosome segregation in cells with intact condensin rings, which achieved a maximum of 76% segregation within the duration of the time course (Figure S3C–S3E). We conclude that condensin ring reclosure after exit from G1 phase is able to rescue chromosome arm segregation to a considerable degree.

Finally, we asked whether condensin ring reclosure at the transition from metaphase to anaphase would be sufficient to prevent chromosome arm missegregation. We cleaved condensin in metaphase-arrested cells (Figure S3F), then released the cells from the arrest, and scored segregation of chromosome V arms (Figures 4E and 4F). Without addition of rapamycin, only 35% of cells segregated the labeled chromosome arm equally between the daughter cells. When we added rapamycin shortly before releasing cells into anaphase, the fraction of cells that correctly segregated chromosome V arms increased to 58% (Figure 4F). For comparison, up to 76% of cells with intact condensin rings segregated chromosome V arms equally in this experiment, independent of whether we had added rapamycin or not (Figures S3F–S3H). Reclosure of cleaved condensin rings shortly before anaphase onset is hence sufficient to restore the correct segregation of long chromosome arms in a substantial fraction of cells.

DISCUSSION

The nature and consequences of the segregation failures that are hallmarks of cells devoid of condensin function have

remained poorly understood. Our live-cell imaging experiments suggest that, following condensin release from chromosomes by opening its ring structure, cells proceed through and exit from mitosis with similar timing as do cells with intact condensin (Figure 3). However, upon completion of cytokinesis, cells with cleaved condensin accumulate chromosome breaks (Figure 2) and consequently arrest in a DNA damage checkpoint-dependent manner (Figure 1). These findings suggest that trailing chromosome arms, which fail to clear the cytokinetic cleavage plane when condensin is not bound to them, are severed either by the forces generated by the contracting cleavage furrow or during abscission. We hypothesize that condensin's primary function might therefore be to tighten chromosome arms, potentially by creating topological linkages (Cuylen et al., 2011), to ensure that the arms are moved all the way to the cell poles during anaphase. Remarkably, restoring condensin topology just before anaphase onset is sufficient to allow proper chromosome arm segregation in a considerable fraction of cells (Figure 4F). If condensin acts as an intrachromosomal linker molecule, then it must be possible that functional links form *de novo* at the time of metaphase.

The discovery that nonsegregated chromosomes are broken during cytokinesis is surprising because we expect that chromatin trapped in the cleavage furrow should be detected by the NoCut pathway, which was suggested to delay abscission and thereby allow completion of segregation and prevent chromosome breakage (Mendoza et al., 2009; Norden et al., 2006). Although cytokinesis and entry into the next cell cycle might be minimally delayed in cells with cleaved condensin rings (Figures 1D and 3A–3H), any such delay is obviously insufficient to prevent chromosome breakage. Our findings are consistent with the formation of DNA breaks in yeast cells that are prevented from completing chromosome segregation by topo II depletion (Baxter and Diffley, 2008) or from resolving sister rDNA loci after Cdc14 inactivation/reactivation (Quevedo et al., 2012). Because the latter results in a delay in karyokinesis, it might have been conceivable that chromosome-bound condensin is required for activation of the NoCut pathway. However, our finding that cells complete cytokinesis irrespective of whether chromosome segregation had been prevented as a result of condensin cleavage, a failure to resolve cohesin, or topo II inactivation (Figure 3K) argues against this hypothesis.

Even though cytokinesis-induced DNA breaks are detected already during G1 phase (Figure 2F), cells proceed through S phase and arrest only in the next metaphase, similar to cells that fail to disjoin chromosome XII (Quevedo et al., 2012). Why do these breaks not trigger a G1/S or intra-S phase DNA damage checkpoint (Longhese et al., 1998)? One possibility is that only very few chromosome arms might get severed during cytokinesis, which could be insufficient to set off a pathway response before G2/M (Zierhut and Diffley, 2008). This is consistent with the finding that the gross of chromosomes in condensin mutants appears intact in pulsed-field gel electrophoresis analyses

or 30 s (G1 phase release; strains C3246 and C3233) after photoconversion. In addition, fluorescence intensity ratios were measured of cells released from a G1 phase arrest after overexpression of a noncleavable version of Scc1 (strain C3144) or of *top2-4* mutant cells (strain C3145) released from a metaphase arrest at the restrictive temperature. Mean intensity ratios (S/N) \pm SD are shown ($n = 21$ –45 cells). See also Figure S2 and Movies S3 and S4.

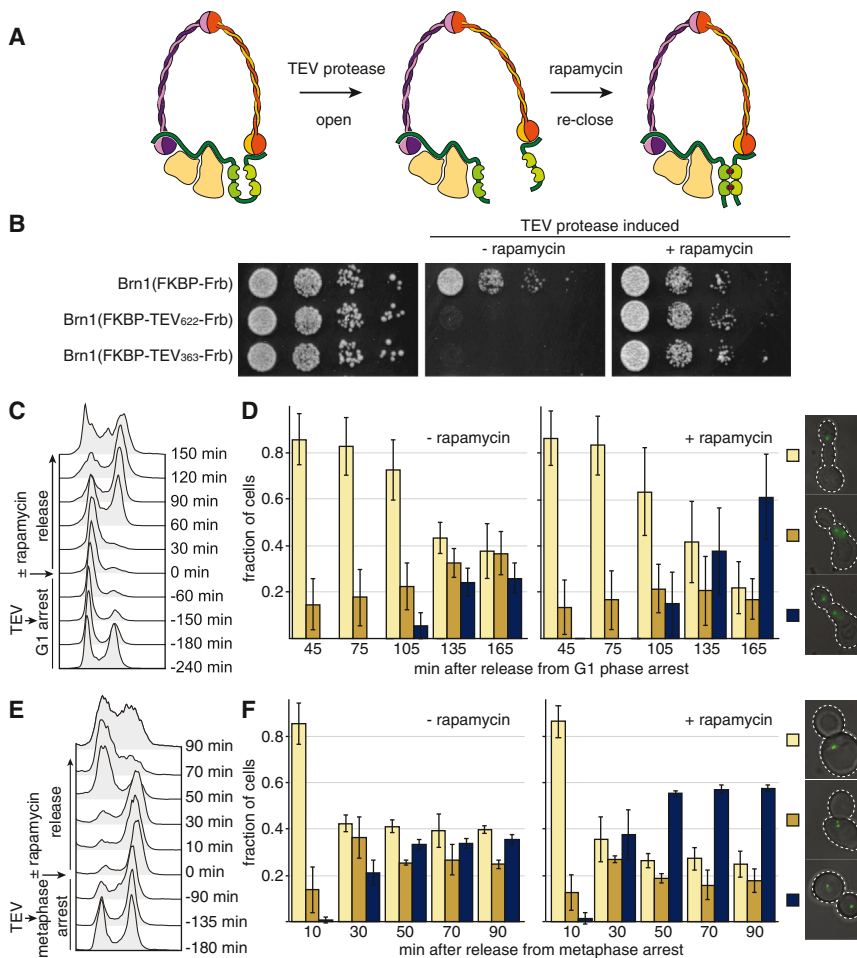


Figure 4. Condensin Ring Reclosure Restores Chromosome Arm Segregation

(A) Condensin rings were opened by site-specific cleavage of Brn1 at TEV protease target sites flanked by two FKBP and Frb domains. Addition of rapamycin induces FKBP and Frb dimerization and thereby closes the ring structure.

(B) TEV protease expression was induced in asynchronously growing cultures (strains C3671, C3574, and C3572) before plating cells on media with or without rapamycin.

(C and D) TEV protease expression was induced in G1-phase-arrested cells expressing Brn1 (FKBP₂-TEV₆₂₂-Frb₂) (strain C3574) before release into media with or without rapamycin. (C) Cell-cycle progression was monitored by FACScan analysis of cellular DNA content (rapamycin culture). (D) Segregation of a GFP marker array integrated proximal to the right telomere of chromosome V was scored by live-cell microscopy. Data represent the mean of quadruplicate experiments \pm SD from 20–39 cells per experiment.

(E and F) TEV protease expression was induced in metaphase-arrested cells expressing Brn1 (FKBP₂-TEV₆₂₂-Frb₂) (strain C3629) before release into media with or without rapamycin. Cell-cycle progression (E) and chromosome V segregation (F) were scored as in (C) and (D). Data represent the mean of triplicate experiments \pm SD from 39–84 cells per experiment.

See also Figure S3.

Live-Cell Microscopy

Cells were transferred onto glass-bottom dishes (MatTek) coated with concanavalin A (Sigma), allowed to settle for 10 min, and then washed three times with –MET or synthetic complete medium containing 2% raffinose and 2% galactose.

Cells were then covered with the same medium and imaged at the indicated time points at 30°C or 37°C (Figure 2H). To monitor the formation of DNA breaks (Rad52-GFP or Mre11-GFP), a fresh batch of cells was transferred to a new dish for each time point to avoid photoinduced DNA damage. To monitor in parallel segregation of chromosome V in the presence and absence of rapamycin, multiwell microscopy slides (ibidi) were used.

Imaging was performed on a DeltaVision Spectris Restoration microscope (Applied Precision) with a 100 \times NA 1.35 oil immersion objective. To monitor spindle formation, 12 z sections with 700 nm step size and 0.07 s exposure times at 50% transmission of the GFP filter were recorded every 3 min. To reduce light exposure of the *rad9 Δ* strain, 16 z sections with 300 nm step size and 0.07 s exposure times at 32% transmission of the GFP filter were recorded only every 10 min. Rad52-GFP and Mre11-GFP foci were recorded at 12 z sections, and chromosome V GFP arrays were recorded at 15 z sections with 700 nm step size and 0.2 s exposure times at 100% transmission of the GFP filter. To monitor cytokinesis dynamics, GFP and mCherry images were captured with 6 z sections of 1 μ m step size every minute using 2 \times 2 binning. Exposure times were 0.05 s at 32% transmission for GFP and 0.1 s at 50% transmission for mCherry.

Photoconversion Experiments

Cytoplasmic EosFP in a circular spot with 2.1 μ m diameter was photoconverted in one daughter cell by three 10 ms pulses from a 405 nm laser set to 1% intensity on an UltraVIEW VoX spinning disk microscope (Perkin-Elmer). Transmission, red, and green fluorescence images were recorded every second from 1 s before to 30 s after conversion at 50 ms exposure and 5% laser power. To quantify the diffusion between daughter cells,

(Lavoie et al., 2002). A second possibility is that the type of DNA breaks generated might not be capable of eliciting G1 or S phase checkpoints (Barlow et al., 2008). Alternatively, processing by the nonhomologous end-joining (NHEJ) repair machinery might mask the DNA breaks. The latter possibility is, however, unlikely because cells with cleaved condensin that lack the NHEJ component Ku70 still arrest in metaphase (Figure 1J). The only possibility to rescue the broken chromosome ends might therefore be either de novo telomere addition (Kramer and Haber, 1993) or fusion to other chromosomes, with the latter entailing the danger to create breakage-fusion-bridge cycles (McClintock, 1939) that could lead to chromosome structure aberrations similar to those recently described in human cells (Gascoigne and Cheeseman, 2013; Janssen et al., 2011). Strikingly, formation of DNA breaks has also been observed in human cells depleted for the condensin subunit Smc2 (Samoshkin et al., 2012). Condensin's function in preventing damage to trailing chromosome arms might therefore be conserved from yeast to humans.

EXPERIMENTAL PROCEDURES

Yeast Strains

All strains are derivatives of W303; detailed genotypes and culture conditions are listed in the Supplemental Experimental Procedures.

the mean fluorescence signals of the red channel were measured in a circular area of 3.3 μm diameter in the photoswitched cell (S) and the nonconverted cell (N), and the S/N ratio was calculated after background subtraction.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and four movies and can be found with this article online at <http://dx.doi.org/10.1016/j.devcel.2013.10.018>.

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